IN THE SPECIFICATION:

After the title, and before line 1 of the specification, kindly insert the following substitute paragraph:

--This application is a continuation-in-part application of parent application serial No. 08/780,086, originally filed on December 23, 1996, now abandoned., which is based on Japanese application No. 7-352918, filed December 27, 1995--

Kindly substitute paragraph 1 on page 1 with the following amended paragraph:

The invention relates to a nonantigenic stabilizer inducing no anaphylaxis reactions wherein the nonantigenic stabilizer is obtained by specifically decomposing ~~gelatin~~ gelatin or collagen using a collagenase, and to a physiologically active substance stabilized thereby.

Kindly substitute paragraph 2 on page 1 with the following amended paragraph:

Pharmaceutical preparations of various physiologically active substances, particularly proteins, enzymes and vaccine preparations, have been developed for treating and preventing various diseases. A ~~gelatin~~ gelatin or collagen (see Japanese Patent Application Laid-Open No. 54-140715; Japanese Patent

Application Laid-Open No. 1-279843; Japanese Patent Application Laid-Open No. 6-234659; Japanese Patent Application Laid-Open No. 51-16488; Japanese Patent Application Laid-Open No. 60-260523; Japanese Patent Application Laid-Open No. 62-149628; and Japanese Patent Application Laid-Open No. 2-49734) or their decomposition products by acid or heat treatment (see Japanese Patent Application Laid-Open No. 49-109520; Japanese Patent Application Laid-Open No. 54-140715; Japanese Patent Application Laid-Open No. 57-114527; Japanese Patent Application Laid-Open No. 63-307827; Japanese Patent Application Laid-Open No. 6-234659; and Japanese Patent Application Laid-Open No. 54-143197) have been used as stabilizer for the preparations. They are used single or in combination with other common stabilizers. A ~~zelatin~~ gelatin collagen and their decomposition products have become popular as stabilizer for various

Kindly substitute paragraph 1 on page 2 with the following amended paragraph:

On the other hand, a rapid increase of the patients of allergic diseases, referred to as civilizational diseases, has been ever lasting in recent years in Japan as well as countries in Europe and the USA. It is even said that one out of three has some allergic disease now. With such increase of allergic patients as a background, those patients who suffer adverse drug reactions such as anaphylaxis against various physiologically active substances containing a ~~gelatin~~ gelatin or collagen as stabilizer which was thought to have little antigenicity/allergenicity (the patients who have ~~gelatin~~ gelatin -specific IgE antibody)

have recently increased little by little, therefore beginning to make a social problem. In fact, it was not before 1990s that academic reports on these reactions have been seen at times (see Kelso, J. M. et al., Allergy Clin. Immunol., vol. 91, 867-872 and Sakaguchi, M. et al., Infection, Inflammation and Immunity, vol. 26, 48-50). It is an important problem since adverse drug reactions such as anaphylaxis should not be caused by a pharmaceutical preparation of a physiologically active substance originally used for treating and preventing various diseases.

Kindly substitute paragraph 2 on page 2 with the following amended paragraph:

The inventors therefore intensively and repeatedly studied on those derivatives of ~~zelatin~~ gelatin and collagen that show no antigenicity or allergenicity in view of such actuality. As a result, we found that a peptide composite with a molecular weight ranging up to 1,000 which has no antigenicity, maintaining $(Gly\text{-}X\text{-}Y)_n$, and a specific amino acid sequence for collagen, by making a single collagenase or the enzyme immobilized on various carriers directly act on materials containing ~~zelatin~~ gelatin or collagen to perform a specific enzymolysis (see Japanese Patent Application Laid-Open No. 7-82299).

Kindly substitute paragraph 3 bridging pages 2 and 3 with the following amended paragraph:

However, no use of the peptide composite shown by the inventors in Japanese Patent Application Laid-Open No. 7-82299 as stabilizer could be thought since the ~~zelatin~~ gelatin whose molecular weight of not more than about 10.000 was conventionally thought to have little stabilizing effects of urokinase as shown in Japanese Patent Application Laid-Open No. 54-80406. The peptide composite shown in Japanese Patent Application Laid-Open No. 7-82299 also had a problem of limitation in raising the yield due to the narrow range of molecular weight.

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Kindly substitute paragraph 2 on page 3 with the following amended paragraph:

The inventors intensively and repeatedly studied on derivatives of ~~zelatin~~ gelatin and collagen that show no antigenicity or allergenicity, and consequently found that those peptide composites with an amino acid sequence $(Gly\text{-}X\text{-}Y)_n$ that is obtained by a specific decomposition of ~~zelatin~~ gelatin or collagen using a collagenaee and has a molecular weight not more than 20.000 not only show no antigenicity/allergenicity but also have an action to stabilize various physiologically active substances.

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Kindly substitute paragraph 3 bridging pages 3 and 4 with the following amended paragraph:

The nonantigenic stabilizer involved in the present invention therefore is characterized in that it is mainly composed of a peptide whose molecular weight is not more than 20,000 and whose amino acid sequence is $(Gly\text{-}X\text{-}Y)_n$ that is obtained by a specific decomposition of ~~zelatin~~ gelatin or collagen using a collagenase. Particularly, the nonantigenic stabilizer involved in the present invention preferably comprises the peptide composite which is obtained by a specific decomposition of ~~zelatin~~ gelatin or collagen using a collagenase, and contains not less than 70% of peptide whose molecular weight is not more than 20,000 and whose amino acid sequence is $(Gly\text{-}X\text{-}Y)_n$. In particular, the nonantigenic stabilizer involved in the present invention preferably contains at least 85%, more preferably 95% of said peptide to increase nonantigenicity.

Kindly substitute paragraph 3 bridging pages 4 and 5 with the following amended paragraph:

Since the physiologically active substances are generally unstable, often changed during storage with the passing of time and their activity is greatly reduced, components derived from ~~zelatin~~ gelatin or collagen are often added as stabilizer in a practical use as described above. The use of the nonantigenic stabilizer involved in the present invention for the physiologically active substances as stabilizer allows preparing those pharmaceutical preparations of physiologically active substances, etc. that will not cause adverse drug reactions such as anaphylaxis.

Kindly substitute paragraph 1 on page 5 with the following amended paragraph:

There is a fear of antigenicity appearing with even those peptides with an amino acid sequence (Gly-X-Y)$_n$ that are obtained by specific decomposition of ~~zelatin~~ gelatin or collagenase using a collagenase if they have a molecular weight over 20,000. The nonantigenic stabilizer involved in the present invention has a molecular weight which is not more than 20,000. Thus it can be prepared with a higher yield from the same raw material than that with a molecular weight not more than 1,000. The nonantigenic stabilizer involved in the present invention preferably has a molecular weight not more than 10,000 to raise its nonantigenicity. Preferably, the percentage of the peptides whose molecular weight is not over 10,000 in those peptides of the nonantigenic stabilizer involved in the present invention whose molecular weight is not more than 20,000 and whose amino acid sequence is (Gly-X-Y)$_n$ is not less than 90%.

Kindly substitute paragraph 5 bridging pages 5 and 6 with the following amended paragraph:

For starting materials of the nonantigenic stabilizer involved in the present invention, a ~~zelatin~~ gelatin or collagen, particularly the collagen or ~~zelatin~~ gelatin prepared from fresh bones, skin, tendon or cartilage derived from animals including a bovine and pig as raw materials may be used. On this occasion, the degree of purification of the collagen or zelatin is preferably higher, but no degree of the purification is specifically required if the purity or specificity of the collagenase used is excellent or a process for purification can be

Kindly substitute paragraph 2 bridging pages 6 and 7 with the following amended paragraph:

It is a common way of thinking in case of the peptides with several thousands or more of molecular weight that they may generally maintain the antigenicity of the raw material partially at least. In actual fact, as shown in the undermentioned example, activity of approximately 1/15 to 1/50 of the antigenicity of ~~zelatin~~ gelatin remained in case of the products of decomposition of ~~zelatin~~ gelatin which had a molecular weight ranging from 500 to 20,000 that were prepared by treating ~~zelatin~~ gelatin or collagen with acids or heating. Further, the antigenicity as gelatin also remained approximately 1/50 to 1/200 in case of those ~~zelatin~~ gelatin composites obtained by treatment not with a decomposing method by acids or heating but with enzymolysis by a single or mixture of more than two general protease such as pepsin, trypsin, papain, chymotrypsin, pancreatin or actinase. Thus it is supposed that the attempt for lower molecular weight by a simple decomposition may not be sufficient for eliminating the antigenicity of ~~zelatin~~ gelatin, and that there may be a close relation between the elimination of antigenicity and a method for decomposing ~~zelatin~~ gelatin.

Kindly substitute paragraph 1 on page 7 with the following amended paragraph:

A critical point for producing the nonantigenic stabilizer involved in the present invention is the purity of the collagenase used. A collagenase, generally prepared from various bacterial bodies, is sometimes contaminated by other proteases. The much contained impure enzymes results in the decomposition of proteins other than zelatin or collagenase in a raw material or the nonspecific decomposition of ~~zelatin~~ gelatin or collagen itself by the impure enzymes. In this case, the quality of the nonantigenic stabilizer purified may be reduced, thereby causing induction of anaphylaxis. It is therefore necessary to take enough care for the purity of collagenase used as well as the substrate specificity.

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Kindly substitute paragraph 2 on page 7 with the following amended paragraph:

The collagenase may be used in a free form or may be used as an immobilized enzyme in which the collagenase is combined with various carriers by physical adsorption or chemical bonding. As a method for enzymolysis by the collagenase, (a) a batch process, (b) a column process, or (c) a method combining the two may be used. The manufacturing line by the methods (a)-(c) and the form of collagenase used may be freely combined. The manufacture of the nonantigenic stabilizer involved in the present invention may be performed in accordance with the method described in Japanese Patent Application Laid-Open No. 7-82299, and also with other methods. It is rather preferable to select a respective method for ~~zelatin~~ gelatin or collagen as raw material or to select a method suitable for maintaining the specificity or purity of collagenase.

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Kindly substitute paragraph 3 bridging pages 7 and 8 with the following amended paragraph:

More concretely, as an example for the method for manufacturing the nonantigenic stabilizer involved in the present invention, the nonantigenic stabilizer may be manufactured as shown in the example below from ~~zelatin~~ gelatin or collagen as starting material by a bioreactor system with a batch or column process using an immobilized enzyme for better enzyme yield. Specifically, a collagenase may be combined with various carriers, i.e. CHITOPEARL, by physical adsorption or chemical bonding, the complex may be packed in a column for chromatography, and a solution of ~~zelatin~~ gelatin solubilized or a collagen denatured at such a temperature that no decomposition occurs, preferably 40-45°C may be allowed to pass through the column for enzymolysis. The speed of transferring the raw material collagen may be properly selected in accordance with the activity of the immobilized enzyme and the needed degree of decomposition.

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Kindly substitute paragraph 2 bridging pages 8 and 9 with the following amended paragraph:

After dissolving 50 g of highly purified ~~zelatin~~ gelatin (produced by Miyagi Chemical Industries, Ltd.) in 1,000 ml. of 20 mM Tris-HCL buffer solution (pH 7.4)/0.1 M NaCl by heating, the solution was cooled to 50°C. An immobilized

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enzyme was prepared by combining 100 mg of collagenase (produced by Washington, Ltd.; a highly purified product from type IV) with 50 g of CHITOPEARL (Fuji Boseki Co., Ltd.) using two crosslinking reagents. The absorbances at 280 nm were measured before and after the binding so as to calculate a percentage of the collagenase bound to the carrier. The percentage was not less than 99%. When the immobilized enzyme was used, it was packed in a tandem column bioreactor with a pH sensor placed between the columns and washed and equilibrated with 20 mM Tris-HCl buffer solution (pH 7.4)/0.1 M NaCl. The system for measuring pH comprises a pH sensor placed between the columns of the tandem columns which senses a change of pH and a tube connected to it from which a concentrated Tris buffer solution flows into it.

Kindly substitute paragraph 2 on page 10 with the following amended paragraph:

[Preparation of ~~zelatin~~ gelatin antiserum (IgG type)]

~~Zelatin~~ Gelatin derived from bovine skin and pig skin was dissolved in PBS to adjust the concentration to 2 mg/mL and the solution was filtered with a 0.22 μm filter. An emulsion was prepared by mixing the same volumes of the filtrate and the Freund's complete adjuvant and injected to three rabbits, 1 mL each. After three weeks, an emulsion was prepared by mixing the same volumes of the solution of same peptide composite and the Freund's complete adjuvant and similarly injected to the rabbits. The procedure was repeated three times, and atisera were obtained on the seventh day after the last immunization.

Kindly substitute paragraph 3 bridging pages 10 and 11 with the following amended paragraph:

[Preparation of ~~zelatin~~ gelatin antiserum (IgE type)]

~~Zelatin~~ Gelatin derived from bovine skin and pig skin was dissolved in PBS to adjust the concentration to 2 mg/mL and the solution was sterilized by filtration with a 0.22μm filter.

B17 Aluminum hydroxide (Alum) was added to the

iltrate as precipitant, and the precipitate was washed and dissolved to prepare a 100μg/mL solution, and the solution was subjected to intracutaneous injection to three guinea pigs, 1 mL each. After 4 weeks, additional immunization was similarly performed, and antisera were obtained after further 3-5 days.

Kindly substitute paragraph 1 on page 11 with the following amended paragraph:

[Preparation of ~~zelatin~~ gelatin sensitized immuno ball]

A ~~zelatin~~ gelatin sensitized immuno ball in whch ~~zelatin~~

B18 gelatin derived from bovine skin or pig skin was immobilized on an aminated polystyrene ball (produced by Sumitomo Bakelite Co., Ltd.) activated by two crosslinking agents and blocked by bovine serum albumin or a surface active agent.

Kindly substitute paragraph 2 on page 11 with the following amended paragraph:

Respectively 200μm of i) The peptide composite with not more than about 20,000 of molecular weight obtained in the example (the nonantigenic stabilizer of the example), ii) the peptide with not more than 1,000 of molecular weight obtained in the example (the nonantigenic stabilizer of the example), iii) partially decomposed ~~zelatin~~ gelatin with molecular weight ranging from 200 to 7,000 obtained by thermolysis (comparative example), iv) enzymolytic ~~zelatin~~ gelatin with molecular weight ranging from 500 to 12,000 prepared by enzymolysis with trypsin and pepsin (comparative example) and v) ~~zelatin~~ gelatin (comparative example) were added to a ~~zelatin~~ gelatin sensitized immuno ball, then 200 μL of either said rabbit ~~zelatin~~ gelatin antiserum of IgG type or said guinea pig ~~zelatin~~ gelatin antiserum of IgE type was added and the mixture was allowed to react at 37°C for 30 minutes to perform competitive reactions of the respective components of i) to v), in the reaction system of antiserum and ~~zelatin~~ gelatin antigen.

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Kindly substitute Table 2 on page 12 with the following amended Table 2:

[Table 2]

Sample (treating method)	Molecular weight	Inhibition rate (%)
① Stabilizer of example	≦ 20,000	0
② Stabilizer of example	≦ 1,000	0
③ Thermolytic ~~zelatin~~ gelatin	200 - 7,000	8.1
④ Trypsin/pepsin enzymolytic ~~zelatin~~ gelatin	500 - 12,000	0.6
⑤ ~~Zelatin~~ Gelatin	(-)	100

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Kindly substitute the paragraph on page 13 with the following amended paragraph:

Using a sterilized physiological saline, a 1/2 serial dilution (1/10, 1/20, 1/40, 1/80 and 1/160) of guinea pig anti-bovine ~~zelatin~~ gelatin antiserum was prepared and 50 μL of respective diluted serum solutions were intracutaneously injected to the back of two SD rats each (male, 8 weeks of age; four rats in total) whose back hair was clipped. After 24 hours, 1.0 mL of a 0.6% Evans blue solution containing 1 mg of the peptide composite with not more than about 20,000 of molecular weight obtained in the example (the nonantigenic stabilizer of the example) was injected to the caudal vein of one of the rats. Similarly, 1.0 mL of a 0.6% Evans blue solution containing 1 mg of bovine zelatin was injected to the caudal vein of the second SD rat as a positive control for the peptide composite in ii). After 60 minutes, all the four rats were sacrificed and the back skin was peeled to observe purpura and measure the size. When the size equaled to, or more than 10 mm, its judgment was (++) or (+++). When the size ranged from 9 mm to 5 mm or 4 mm to 1 mm, the judgments were respectively (+) and (±). When no purpura appeared, the judgment was (-). The results are shown in Table 3. From the results in Table 3, no purpura appears even with 1/10 serial dilution of the peptide composite with not more than about 20,000 of molecular weight obtained in i) the example, showing that the composite has no antigenicity, while ii) bovine ~~zelatin~~ gelatin causes purpura even with the 1/160 serial dilution.

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Kindly substitute Table 3 and the paragraph on page 14 with the following amended Table 3 and paragraph:

[Table 3]

Sample	Antiserum serial dilution	Judgment of results of PCA reaction
Bovine ~~zelatin~~ gelatin	1/10	(++ - +++)
	1/20	(++)
	1/40	(+)
	1/80	(+)
	1/160	(±)
Stabilizer of example	1/10	(-)
	1/20	(-)
	1/40	(-)
	1/80	(-)
	1/160	(-)

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Using sera (containing ~~zelatin~~ gelatin specific IgE) collected from six patients showing allergy against ~~zelatin~~ gelatin (Patient A to F in Table 4) and the ~~zelatin~~ gelatin sensitized immuno ball shown in example 7, the degree of inhibition of the titer of ~~zelatin~~ gelatin specific IgE antibody by the nonantigenic stabilizer of the example by measuring the fluorescence of fluorescent substrate decomposed by the labeled enzyme on performing the fluorescent enzyme immunoassay to measure the ~~zelatin~~ gelatin specific IgE antibody that of a patient who was found positive by fluorescent enzyme immunoassay. The fluorescence

intensity was measured at 495 nm of excitation wavelength and 520 nm of fluorescence wavelength using a fluorophotometer (FP777; JASCO). A ß-galactosidase labeled mouse anti-human-IgE antibody was used as the second antibody to detect the ~~zelatin~~ gelatin specific IgE antibody, and the enzyme activity was assayed using a fluorescent substrate. The results are shown in Table 4.

Kindly substitute Table 4 and the paragraph on page 15 with the following amended Table 4 and paragraph:

[Table 4]

Sample		Titer of antibody from respective patients of allergy (fluorescence intensity : F.I.)					
Sample name	Molecular weight	A	B	C	D	E	F
Control group : no sample	(-)	8,890	1,542	9,004	6,663	1,034	8,226
Stabilizer of Example	≦20,000	8,778	1,608	8,905	6,560	1,029	8,007
Stabilizer of Example	≦ 1,000	8,995	1,621	9,550	6,696	1,150	8,544
~~Zelatin~~ gelatin	(-)	267	59	822	85	41	307
Thermolytic gelatin	200-7,000	594	115	978	1,601	(-)	(-)
Trypsin/pepsin enzymolytic gelatin	500-12,000	1,007	105	855	1,025	(-)	(-)

As shown in Table 4, no reduction of the titer (fluorescence intensity) of antibody by inhibitory reaction was observed with the peptide with not more than about 20,000 of molecular weight and the peptide with not more than about 20,000 of molecular weight obtained i the example (the nonantigenic stabilizers of the example), revealing that the peptides have no reactivity with a ~~zelatin~~ gelatin specific IgE antibody. On the contrary, it wa shown that a strong inhibition was caused by the original raw material ~~zelatin~~ gelatin, thermolytic ~~zelatin~~ gelatin or ~~zelatin~~ gelatin decomposed by a non-specific protease and they reacts well with a ~~zelatin~~ gelatin specific IgE antibody, revealing one of the cause for anaphylaxis. Performing the test by the example may inform you whether respective peptide composites have reactivity with the ~~zelatin~~ gelatin specific IgE which induces type I allergy or not.

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Kindly substitute the paragraph bridging pages 18 and 19 with the following amended paragraph:

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According to the nonantigenic stabilizer involved in the present invention, no anaphylaxis reactions are induced due to eliminated antigenicity

as well as characteristics of amino acid sequence of ~~gelatin~~ gelatin or collagen, thereby advantageous for a stabilizer of physiologically active substances for treatment and prevention. According to the nonantigenic stabilizer involved in the present invention, a wider range of molecular weight than that of conventional non-antigenic peptide composites can be provided, and the yield can be increased.

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